S&TR July/August 2018

The Case for Modern Forensic Science

Armed with wide-ranging expertise and analytical capabilities, scientists at Livermore's Forensic Science Center are prepared for anything that comes through its doors.

Chemist Brian Mayer cleans a source component of a mass spectrometry system. The Forensic Science Center (FSC) relies on a suite of complementary instrumentation to meet proficiency requirements for multiple agencies. (Photo by George Kitrinos.)

SUCCESS in a high-stakes field does not come easily. Last fall, Lawrence Livermore's Forensic Science Center (FSC) completed another grueling 15-day testing period for the Organisation for the Prohibition of Chemical Weapons (OPCW), the international body that oversees compliance with the Chemical Weapons Convention treaty. With the ability to detect evidence of chemical warfare agents (CWAs) both as intact chemicals and through their degradation products, the FSC has earned its standing among OPCW-certified laboratories in the United States as one of only two that analyzes environmental samples and one of three that determines human exposure to CWAs. (See *S&TR*, June 2013, pp. 13–15.)

OPCW's proficiency tests challenge the world's best forensic laboratories in technical skills, analytical methodologies, safety protocols, chain-of-custody procedures, and more. Test samples can be made of any material spiked with unknown chemical compounds at varying concentrations. A single mistake—failure to identify spiking compounds, false-positive results, or misreported data—can strip a laboratory of its accreditation. For the eighth consecutive test, the FSC came out on top with an "A" grade.

"These proficiency tests push the limits of our team, technologies, and technical expertise. The system we have developed over the years to enable success across these testing areas has become foundational to much of the work we execute at the FSC," says the center's director Brad Hart. According to Glenn Fox, former FSC director and now associate director for the Laboratory's Physical and Life Sciences Directorate, "The vision for the FSC was ahead of its time. The Laboratory saw a need for, and invested in, its unique capabilities."

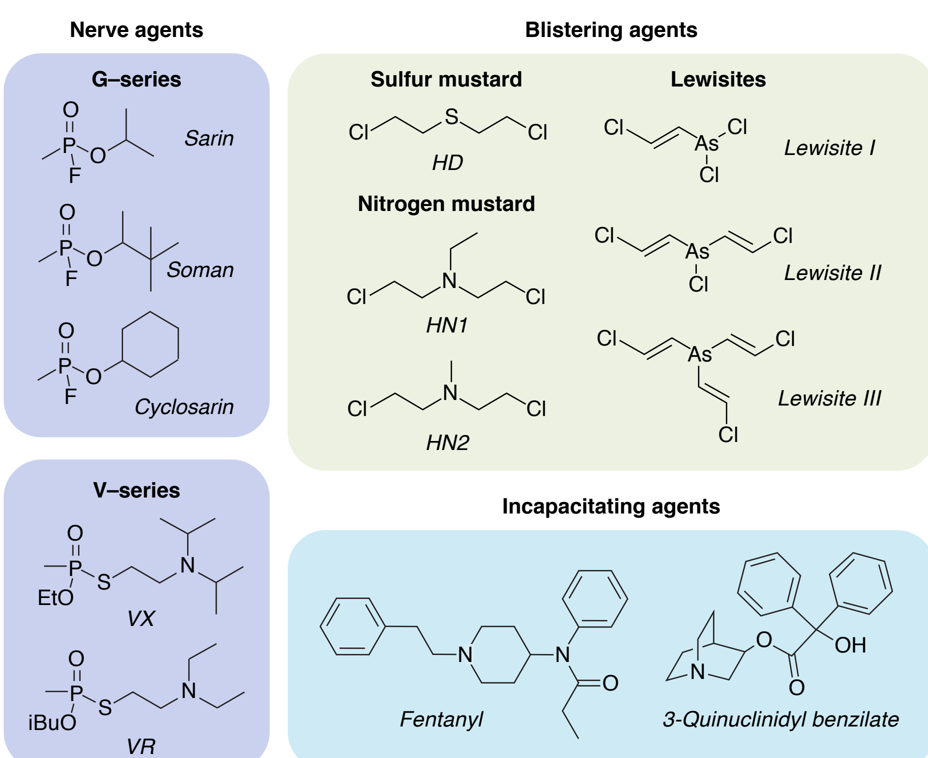
As the scientific community questions the reliability of traditional forensic science in law enforcement investigations, the FSC provides leadership in advancing conventional methodologies through its accuracy in chemical, biological, radiological, nuclear, and explosives (CBRNE) forensic analyses and its research and development efforts in analytical techniques. Bruce Warner, Livermore's principal associate director for the Global Security Principal Directorate, states, "The Forensic Science Center has been providing a balanced operational and research portfolio for 27 years. Government sponsors rely on the center's deep technical capabilities for sample analysis and origin determination and for development of new techniques to address emerging threats."

Inflection Point

In 2016, the U.S. President's Council of Advisors on Science and Technology (PCAST) released a report recommending better scientific standards for forensic methods, specifically those used to compare different forms of evidence such as firearm ballistics, bitemarks, and hair, among others. The document drew on numerous papers, criminal cases, and reports from other federal agencies to capture the scope of the current techniques' shortcomings. PCAST concluded that methods reliant on "significant human judgment" are too subjective and, therefore, not scientifically defensible in court.

The PCAST report built on an earlier study from the National Research Council regarding the state of forensic science, and the cumulative scrutiny leads to what Hart calls an inflection point in transforming this branch of the discipline. He explains, "Inadequate techniques undermine the criminal justice system and increase resistance to their use in the broader national security community. We see opportunities to improve objectivity in this area because of our focus on advancing capabilities that generate high-quality, defensible data."

The FSC is already making strides in traditional forensic analysis with projects that expand the possibilities for human identification. Hart states, "Lawrence Livermore has a unique opportunity to influence this field. Few places can offer what we provide—extensive basic science research that informs our development of technologies and capabilities for application to real-world scenarios."



Categories of chemical warfare agents (CWAs) include nerve, blistering, and incapacitating agents. The FSC's chemical forensic work includes characterizing the constantly evolving range of CWAs.

Strict Science and Safety

One of the FSC's key functions is advancing science in the public interest, and the center's staff thrive on the challenge of growing its forensic capabilities. "We like to push technology in new directions. For example, we consider how next-generation versions of instruments, such as mass spectrometers, will affect what we can detect in the environment," says Carolyn Koester, the center's deputy director for operations. FSC colleague and chemist Brian Mayer adds, "The center joins sophisticated equipment with multidisciplinary expertise in the same physical laboratory space. This synergy is critical to our continual modernization." (See the box on p. 10.)

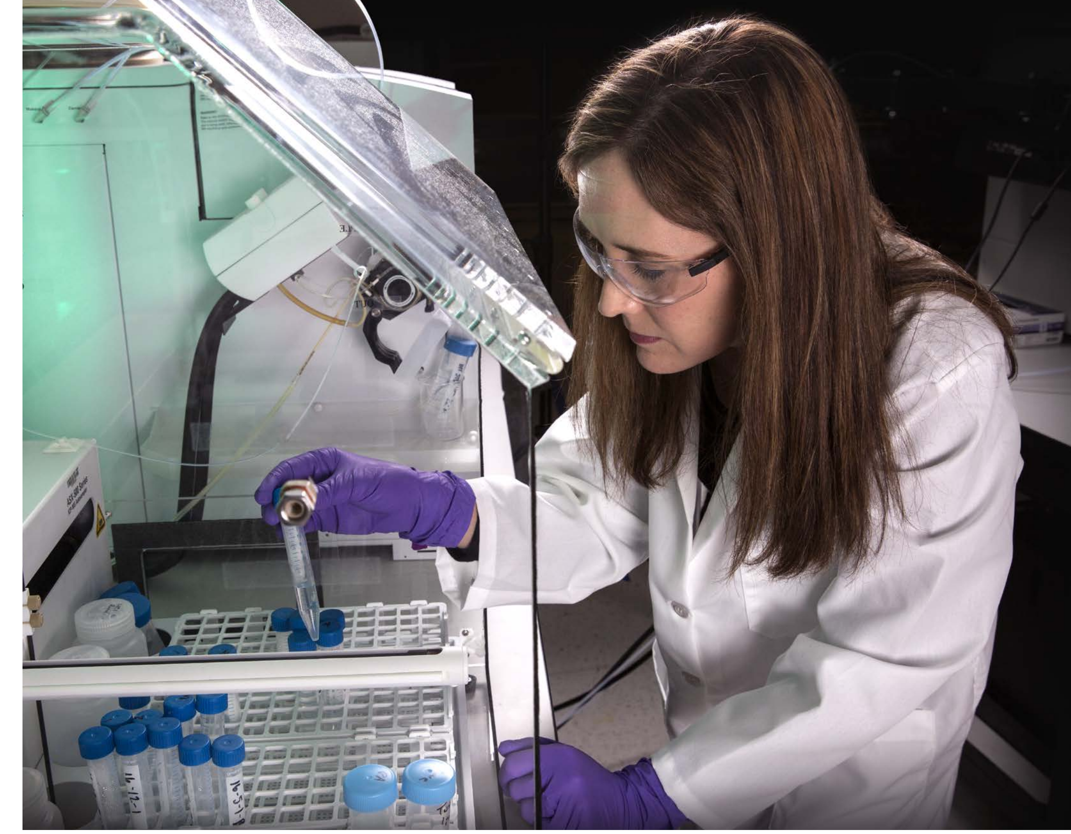
Although most work done at the FSC is performed by in-house staff, the depth and breadth of Livermore's expertise across the complex enables investigators from other areas to help conduct diverse examinations. For example, a specialist from another area of the Laboratory may participate in a specific project, or another facility may provide access to complementary equipment. Chemist Pat Grant says, "We can 'borrow' scientists from other Laboratory programs for an integrated evaluation, thanks to the scientific and technical diversity onsite."

Safety is ingrained in all FSC activities. A strict acceptance procedure ensures proper triage, routing, and screening of materials while minimizing human exposure and sample contamination. Biological materials are handled in a dedicated biosafety area, and the cradle-to-grave custody process includes decontamination and proper disposal of all samples after analysis. "Inappropriate handling could result in severe consequences," notes Koester. "Until we confirm what the specimen is, we treat all samples with the highest precautions," says FSC deputy director Audrey Williams.

When needed, the center runs 24 hours a day, 7 days a week, and staff are dedicated to optimum and efficient turnaround. "We address real-time problems and think comprehensively across the forensic landscape—an attribute that our external partners value," explains Hart, citing the FSC's long-standing chemical attribution signatures program with the U.S. Department of Homeland Security. Also, as a partner laboratory with the Federal Bureau of Investigation (FBI), the Laboratory's FSC analyzes special nuclear materials and various chemical threats. The center's relationship with the FBI became codified after the September 11, 2001, terrorist attacks. Grant is the principal investigator for the center's FBI casework, which includes analysis of weapons-grade materials and other questioned samples. He notes, "When the FBI needs chemical or nuclear forensic capabilities for uncommon investigations, they often come to us."

The C in CBRNE

Chemical threats account for a significant portion of the FSC's investigations, so research and development efforts include synthesizing small amounts—as allowed under the Chemical Weapons Convention treaty—of chemical warfare agents to understand possible production methods clandestine actors may use. For example, FSC scientists have studied multiple pathways for making sulfur mustard, also known as mustard gas. "We look for data that make a given synthesis route or the final product unique," explains Williams. This work informs investigations by comparing a sponsor's questioned sample to idealized materials. Furthermore, comparing samples obtained by different sponsors may reveal whether they are related.



Forensic chemist and FSC deputy director Audrey Williams loads prepared samples into the FSC's inductively coupled plasma–mass spectrometry system, which is commonly used to analyze a sample's trace metal components or contaminants. Beforehand, samples undergo acid digestion to remove organic materials while generating and retaining metal ions. (Photo by George Kitrinos.)

The FSC's chemical forensic expertise extends to chemical attribution. By building profiles or "signatures" of different CWAs, FSC staff can determine important information about a sample. A chemical signature is defined as a collection of impurities, including reaction byproducts, degradation products, and unreacted starting chemicals and reagents and their relative concentrations, detected in a final synthetic product. These chemical signatures are used to provide clues to how the sample was synthesized.

According to Williams, the FSC approaches each research question and chemical sample as a puzzle to be solved. "Our research, development, and operational missions strengthen each other because we have built a collective knowledge base," she says. For instance, the center's CWA detection strategies for OPCW proficiency tests underpin everyday work identifying chemical signatures and characterizing chemical threats. Williams continues, "We provide expert-level evaluation of our measurements, not just the raw data. We go beyond simply reporting that certain chemicals are present in a sample to pull all the clues together in context. Essentially, X and Y together may suggest Z is occurring."

The B in CBRNE

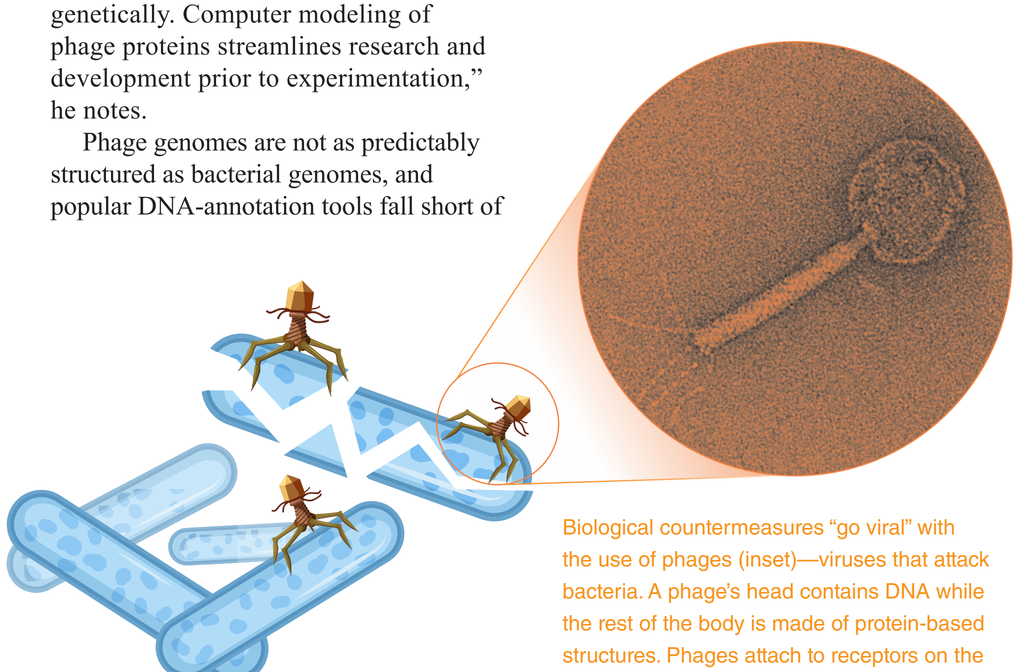
The FSC's success in biological forensic analysis springs from a robust cycle of research followed by applied proof-of-concept studies; partnerships with Livermore's Biosciences and

Biotechnology Division as well as other laboratories; and collaborative scale-up, testing, and deployment phases. Brian Souza, senior biologist and group leader for Biosecurity and Bioforensics, describes this process as unique. “Our biologists think outside the box. In addition, we have access to select agents, including dangerous pathogens,” he says. Souza’s team works closely with the Department of Defense, its Defense Threat Reduction Agency, and other agencies to advance development of biological countermeasures using Livermore’s capabilities in bioinformatics and computational biology.

Biological countermeasures neutralize or reverse physiological responses to naturally occurring and engineered pathogens. To fight antimicrobial resistance, FSC scientists are exploring the potential of viruses that attack bacteria—known variously as phages, bacteriophages, or microbial viruses. Souza’s team and collaborators are developing new methods for characterizing phage genomes. “We need to understand how viruses are organized genetically. Computer modeling of phage proteins streamlines research and development prior to experimentation,” he notes.

Phage genomes are not as predictably structured as bacterial genomes, and popular DNA-annotation tools fall short of identifying phage genes. Using Livermore’s high-performance computing resources, the team has designed a one–two punch for conducting the required analysis. First, PHANOTATE, a novel computational algorithm, identifies genes by maximizing their translatable parts—called open reading frames—within the phage’s DNA. Next, the Python-based Phage Annotation Toolkit and Evaluator (PhATE) program uses PHANOTATE’s results to predict protein structure and function encoded in the open reading frames. The combination of PHANOTATE and PhATE enables researchers to determine and model genes that when translated into proteins are used during infection and destruction of their host cell. Understanding these mechanisms of action may help researchers discover better and more effective protein-based countermeasures and antimicrobials.

Beyond national security and public health applications, research into viral DNA markers can guide further forensic endeavors. Souza explains, “Informatics tools enable us to assemble DNA sequences faster, so the methods we develop for biological studies can be shared to benefit human forensic work that uses DNA.” In addition, a microbe’s epigenetic DNA changes (those not related to DNA sequencing) and its genetic markers based on environmental exposure can inform studies of similar mechanisms in human proteins. Souza adds, “Our center-based approach to forensic science is crucial. We have several boutique capabilities that, along with investments and skilled partners, allow us to move quickly in this space.”



Biological countermeasures “go viral” with the use of phages (inset)—viruses that attack bacteria. A phage’s head contains DNA while the rest of the body is made of protein-based structures. Phages attach to receptors on the surface of a bacterium, penetrate the cell, and replicate their DNA, ultimately destroying the cell.

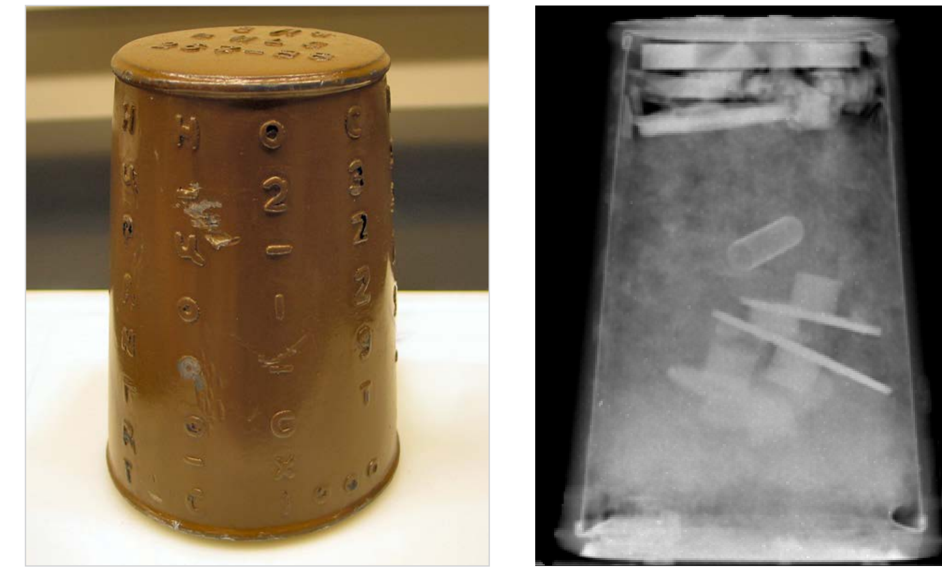
The R, N, and E

Before the FSC was formally established, Livermore radiochemists measured nuclear and radioactive materials resulting from nuclear explosive testing. The 1990s saw a worldwide increase in material seizures, and since then, FSC scientists have been at the forefront of nuclear forensics and attribution, helping to deter trafficking of illicit materials. (See *S&TR*, October/November 2014, pp. 12–18.) Grant remarks, “We have learned much more from comprehensive analyses of real-world interdictions and samples than from scripted exercises or fabricated test specimens.” This accumulated experience has produced a primary reference source for the nuclear forensic field. Grant, along with FSC associates Ken Moody and the late Ian Hutcheon, authored the seminal textbook, *Nuclear Forensic Analysis*, now in its second edition.

Different aspects of the center’s CBRNE capabilities often dovetail with surprising results. In the FSC’s first high-profile case, a controversial nuclear energy experiment at a California research laboratory caused a fatal explosion. Debris analysis revealed only natural background radioactivity, but further investigation uncovered another potential explosion initiator unrelated to the original hypothesis. The case underscores the importance of comprehensive investigation—an imperative for objective forensic analysis—and reinforces the need for access to a variety of skills and techniques.

When conducting an investigation, FSC scientists must consider both principal and collateral aspects of the work at hand. For explosives, such analysis means identifying impurities and additives within explosive compounds, any residues, and the composition of the primary container. For interdicted radiological or nuclear materials, a complete evaluation may also include microscopic analysis of packaging materials and fibers, biological traces left by the traffickers, and other substances such as dusts or pollens, which could provide route and storage information.

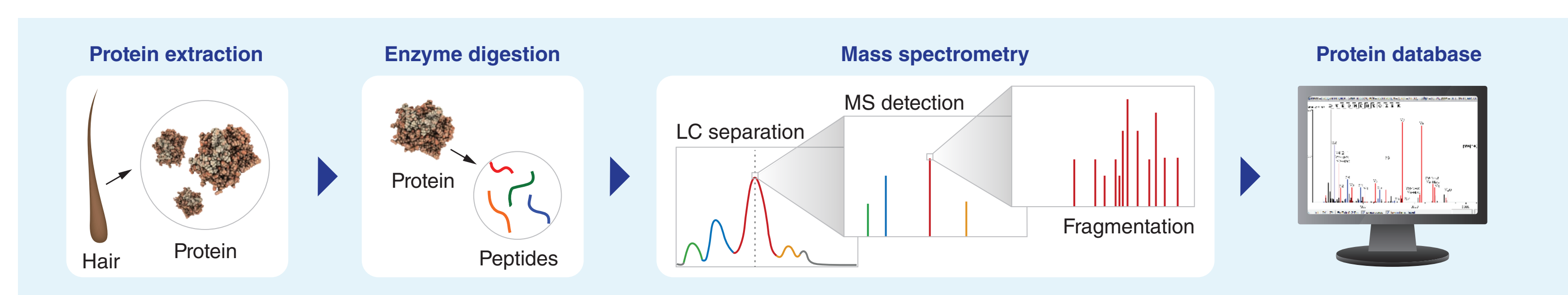
This careful approach means FSC scientists can uncover a hoax just as well as revealing a real threat. In one notable investigation, two containers advertised as smuggled nuclear materials were acquired. Despite being labeled as containing uranium and plutonium, neither object held radioactive or weapons-grade materials. FSC scientists, along with colleagues from Livermore’s Nondestructive Characterization Institute, evaluated all aspects of the containers. They began with a series of nondestructive tests, including gamma-ray spectrometry and x-ray and neutron radiography, to interrogate the specimens’ interiors for safety assessment. Ultimately, subsequent forensic analysis determined that the contents were not dangerous.



(left) Shown here is one of two specimens obtained from a nuclear smuggling enterprise that turned out to be a scam. (right) Researchers evaluated all aspects of the container using multiple imaging techniques, such as neutron radiography. After “breaking and entering” the specimen, the team identified an assortment of packaging and filler materials, including plant matter, pharmaceutical compounds, synthetic fibers, and magnetic objects. (Photo by Pat Grant; radiograph courtesy of Harry Martz, Bill Brown, Randall Thompson, and John Rodriguez.)

Promising Proteomic Identification

Enhancing objectivity in traditional forensic science requires maximizing the potential of tiny pieces of evidence, such as a skin cell or bone fragment. Proteins are a viable source of human identification because they can express DNA mutations and are found in many types of tissue. According to chemist Deon Anex, proteomics can augment DNA analysis in degraded samples such as human remains. He says, “Proteins are more robust in the environment than DNA.” In a recent collaboration, FSC scientists developed a technique for biological identification that uses proteins found in human hair. (See *S&TR*, July/August 2015, pp. 15–17.) Anex and Hart are now leading an effort to expand this work to other human tissues.



Proteomic analysis of hair protein begins with an extraction process that dissolves the hair and isolates the peptide chains. Liquid chromatography–mass spectrometry (LC–MS) is used to separate the peptides and analyze their amino acid sequences.

Top-Notch Technology

The instrument catalog at Lawrence Livermore's Forensic Science Center (FSC) reflects the variety of highly specialized work the center's scientists perform. These investments help ensure that forensic analysis remains focused on defensible data. According to Carolyn Koester, the FSC deputy director of operations, "The center acquires and maintains modern technology to stay at the forefront of forensic science. We have access to equipment not readily available elsewhere." Altogether, FSC operations comply with the International Organization for Standardization's competency and calibration requirements, collectively known as ISO 17025.

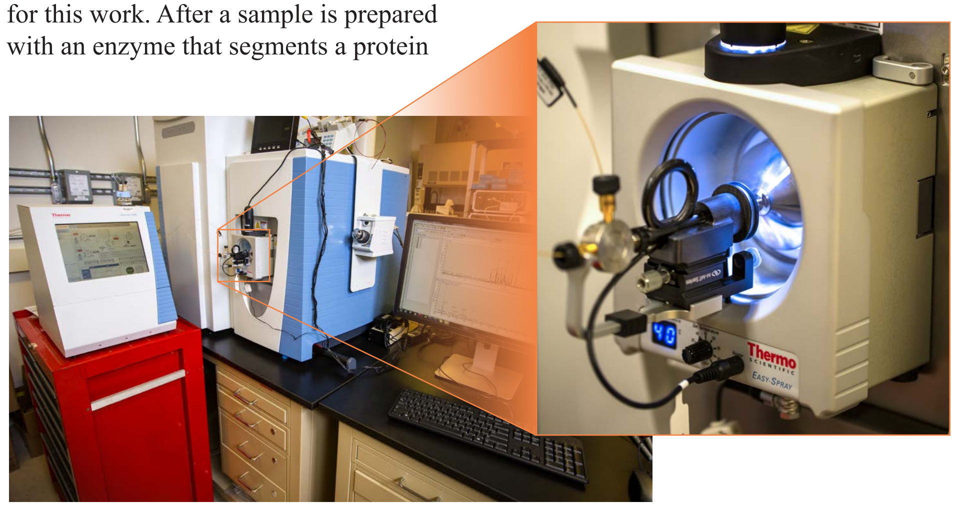
Most of the FSC's tools are co-located in approximately 4,000 square meters of laboratory space, though others are available in nearby Lawrence Livermore facilities. "We can address a wide range of problems in a small space," notes chemist Brian Mayer. Infrared, Raman, and nuclear magnetic resonance spectroscopies provide a wealth of chemical information without damaging samples. X-ray, gamma-ray, and alpha-particle spectrometries support identification and quantitative analyses of materials undergoing radioactive decay.

Many configurations of mass spectrometry (MS) are crucial for identifying and characterizing trace amounts of materials, chemicals, and elements. Koester states, "Measuring mass easily and accurately in complex matrices is a game changer. MS is the common denominator in much of our work." Inductively coupled plasma–mass spectrometry (ICP–MS) has excellent sensitivity over the element range from lithium to the actinides. In ICP–MS, a high-temperature plasma converts the atoms of the elements in the sample to ions. The masses of the ions are then measured, and concentrations of elements below 1 part per billion can be detected.

While ICP–MS detects inorganic compounds, MS coupled with either gas chromatography (GC) or liquid chromatography (LC) detects organic chemicals. Sample introduction into the MS system by GC or LC allows many hundreds of compounds in a sample to be separated as they move through a column, enabling identification of each of these substances by their unique retention times within the column in conjunction with their mass spectra. GC–MS is valuable for analyzing small organic compounds, such as toxic industrial chemicals and chemical warfare agents. LC–MS is used for the detection of large molecules, including biomolecules that indicate human exposure to chemical agents and the proteins and peptides at the heart of the FSC's proteomics work.

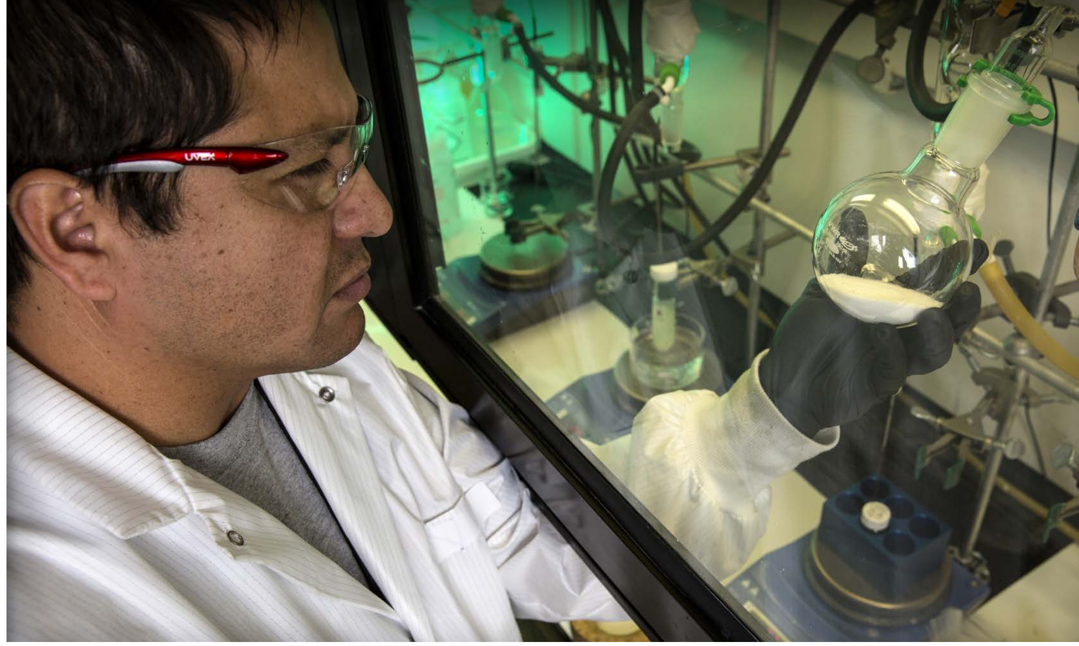
Funded by the Laboratory Directed Research and Development Program, FSC scientists are refining identification methods to use a single hair instead of a bulk sample while also increasing the diversity of the data—that is, incorporating hair from multiple ethnic groups. The project also includes development of an extraction technique for analyzing mitochondrial DNA in hair and for identifying genetically variant peptides in bone, teeth, and skin cells from fingermarks or palmprints. "The techniques we have developed for hair can be modified to use with skin cells," notes Anex. Furthermore, amelogenin, the protein found where a tooth's enamel and dentin meet, contains markers for X and Y chromosomes. Thus, an individual's sex could be determined from analysis of teeth alone, aiding in human identification.

Anex's team draws on the FSC's stable of sophisticated equipment to "read" a full protein sequence. Liquid chromatography–mass spectrometry (LC–MS) is an effective divide-and-conquer strategy for this work. After a sample is prepared with an enzyme that segments a protein chain into multiple peptides, LC's high-pressure liquid flow separates thousands of peptides. Each peptide is then fragmented using MS, and the resulting mass spectrum helps determine the sequence of amino acids. Sequence variations observed in these peptide profiles can be used to identify individuals.



FSC's LC–MS system can measure the mass of a peptide in a sample weighing less than 1 microgram with extremely high accuracy. (inset) The nanospray interface injects separated peptides into the MS component as they elute from the LC unit at a rate of 300 nanoliters per minute. (Photos by George Kitrinos.)

The FSC's proteomics work continues to grow thanks to the Intelligence Advanced Research Projects Activity, which funds research across many scientific fields for the U.S. intelligence community. Anex and colleagues are building a database of protein mutations for individualized human DNA sequences with an eye on operational applications. In the future, crime laboratories could use a protein analysis kit that contains population-dependent reference standards for peptide markers, enabling faster evidence evaluation. Other applications include identifying disaster victims and creating genetic histories from archeological specimens. Anex says, "FSC scientists are using forensic analysis tools in ways the conventional proteomics community is not. We are excited to continue developing these methods for more specific and practical analyses."



FSC scientist Carlos Valdez examines the product of a chemical synthesis. (Photo by George Kitrinos.)

Ready for Anything

FSC staff have come to expect the unexpected. Grant, who served for 15 years as the center's first deputy director, says the future of the field's constantly changing environment is difficult to predict. "Real-world CBRNE samples and scams generally throw something new at us regardless of our experience level," he states. So, with traditional forensic applications in mind, FSC scientists are increasing the reliability and accessibility of CBRNE forensic analyses.

One forward-looking project uses machine learning for statistical analysis of chemical attribution signatures. "Statistics can provide a wealth of chemical insight into a given sample, and machine learning offers new mechanisms for deep, objective data analysis," explains Mayer. The team is testing what a computer program can "learn" from data on synthesis routes of the opioid 3-methylfentanyl (3MF). Certain chemical features of the drug indicate, for example, whether the chemist started with a commercially available material versus a homemade concoction. Recently published results show that the computer program can discriminate among 3MF synthesis routes by identifying the presence or absence of specific impurities in a crude reaction mixture. Eventually, FSC staff plan to help make chemical signatures data more relevant to mainstream law enforcement. In fact, Mayer's team is making this analysis field portable by coding the program for use on a simple laptop.

Outside the Laboratory, FSC scientists support the U.S. Department of State's Chemical Security Program (CSP) with specialized CWA training for first responders in other countries. High-priority areas such as Jordan, Lebanon, and Turkey have benefitted from hands-on field experience and tabletop exercises conducted by forensic specialists. FSC staff teach participants about protection protocols, prevention measures for reducing the risk of further contamination, use of screening equipment, and evidence collection. "CSP views us as their chemical threat response and laboratory analysis experts," says Armando Alcaraz, who leads the FSC's CSP efforts in addition to overseeing the center's OPCW work.

Since 2013, the center has worked with response teams all over the world, notably receiving recognition from the Iraqi Ministry of the Interior when CSP-trained responders correctly identified sulfur mustard after an attack in Kirkuk. The multiphase program also helps responders interface with their country's forensic laboratories to establish and maintain analysis protocols. Alcaraz notes, "When new chemical threats emerge, such as improvised agents, we add them to the curriculum."

As technology and threat spaces advance, so do the FSC's capabilities. "The center has become more diversified as times have changed," observes Fox. Hart agrees, "The challenges our sponsors present are constantly evolving. As a result, we must anticipate and adapt to ensure we are ready for anything that comes through the door."

—Holly Auten

Key Words: chemical attribution; chemical, biological, radiological, nuclear, explosives (CBRNE) forensic analyses; Chemical Security Program (CSP); chemical warfare agent (CWA); DNA; forensic science; Forensic Science Center (FSC); gas chromatography–mass spectrometry (GC–MS); human identification; inductively coupled plasma–mass spectrometry (ICP–MS); liquid chromatography–mass spectrometry (LC–MS); machine learning; nuclear forensics; Organisation for the Prohibition of Chemical Weapons (OPCW); phage; Phage Annotation Toolkit and Evaluator (PhATE); proteomics.

For more information contact Brian Mayer (925) 423-1128 (mayer22@llnl.gov).